Reference: *Biol. Bull.* **180:** 496–504. (June, 1991)

# The Induction of Carbonic Anhydrase in the Symbiotic Sea Anemone *Aiptasia pulchella*

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**Abstract.** The activity and nature of carbonic anhydrase (CA, EC 4.2.1.1.) was measured and described in the tropical sea anemone *Aiptasia pulchella.* The hypothesis that high CA activity in animal tissue is induced by the presence of symbiotic algae was tested. CA activity was positively correlated with the number of symbiotic dinoflagellates (zooxanthellae) present. CA activity in aposymbiotic anemone tissue was 2.5 times lower than that in control symbiotic animals or in aposymbiotic animals repopulated with algae. Polyclonal antisera against human CA were used to probe for the presence of CA in both symbiotic and aposymbiotic anemone tissue, and in freshly isolated and cultured zooxanthellae. The resulting immunoblots showed one band with a molecular weight of 30 kDa in symbiotic animal tissue and control mammalian CA lanes, no bands in the aposymbiotic animal lanes, and one band at a molecular weight of 22.5 kDa in freshly isolated and cultured zooxanthellae lanes. Because no 22.5 kDa band was detected in the symbiotic animal tissue lanes, the high CA activity found in symbiotic animal tissue is considered to be due to the induction of animal enzyme by the presence of algae. The lack of any band in the aposymbiotic lanes further supports the hypothesis that CA activity in *A. pulchella* is induced by the presence of algae.

## Introduction

Symbiotic dinoflagellates ("zooxanthellae") residing in vacuoles within cells of marine cnidarians exhibit a high rate of photosynthesis (Falkowski *et al.*, 1984). When this rate exceeds the respiration rate of the association, the algae must draw on inorganic carbon ($C_i$) from the seawater pool to satisfy the high carbon demand. $CO_2$ is the $C_i$ species preferred as a substrate for carbon assimilation by ribulose bisphosphate carboxylase/oxygenase (RUBISCO) in the zooxanthellae. Yet at an ambient pH of 8.2–8.3, $C_i$ in seawater is present mostly as $HCO_3^-$. Additionally, the movement of $HCO_3^-$ across unstirred boundary layers and the several animal and algal membranes to the site of photosynthesis could be relatively slow (Kerby and Raven, 1985).

Weis *et al.* (1989) hypothesize that the supply of $CO_2$ for photosynthesis in algal/cnidarian symbioses is augmented by the presence in the cnidarian tissue of carbonic anhydrase (CA, EC 4.2.1.1.), an enzyme that catalyzes the inter-conversion of $HCO_3^-$ and $CO_2$. In the 22 species of cnidarians examined, CA activity in the animal tissue of symbiotic species was, on average, 29 times higher than in non-symbiotic species. In the symbiotic species, CA activity in the animal fraction was 2–3 times higher than that in the algae. These results suggest that CA activity in animal tissue is related to the presence of zooxanthellae.

Two other findings indicate that CA activity in symbiotic animal tissue is related to the presence of algae. First, CA activity is correlated with habitat irradiance in colonies of the coral *Stylophora pistillata* (Weis *et al.*, 1989). *S. pistillata* from high light habitats exhibited significantly higher rates of CA activity than did those living at lower light levels. Second, there are spatial differences in CA activity within the same individual (Weis *et al.*, 1989). Column tissue of the anemone *Condylactis gigantea*, which lacks symbionts, had very low activity compared to the tentacle tissue which contains symbionts.

In this study I present further evidence, from work on symbiotic and aposymbiotic *Aiptasia pulchella*, of a positive correlation between the CA activity in animal tissue and the number of zooxanthellae present. Additionally, I use the immunoblot technique to show that high CA

Received 2 October 1990; accepted 25 February 1991.

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activity in symbiotic animal tissue is the result of induction in the animal tissue by the presence of the algae.

## Materials and Methods

### *Maintenance of experimental organisms*

A clone of the anemone *Aiptasia pulchella* (Java clone) was maintained in laboratory in aquaria or large finger bowls containing Millipore-filtered seawater (MFSW) obtained from Santa Monica Bay. For at least 14 days prior to experimentation, anemones were kept in a Precision incubator at 25°C at an irradiance of 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ on a 12 h light/dark cycle unless otherwise specified. Throughout the experiments the anemones were fed *Artemia* nauplii once weekly, and the finger bowls were cleaned and the water was changed daily.

Zooxanthellae isolated from the Java clone were grown in ASP-8A medium (Guillard and Keller, 1984) in 25 l clear plastic carboys. The carboys were incubated at room temperature at an irradiance of approximately 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ (16 h light/8 h dark cycle). The cultures were aerated with air passed through a bacterial air filter (Gelman Bacteria air vent). One carboy would yield approximately 10 ml of wet packed cells after approximately 75 days. The cells were collected by centrifugation and stored at −70°C.

### *Aposymbiotic and repopulated animals*

A three part study was designed to measure CA activity in symbiotic, aposymbiotic, and newly repopulated symbiotic animals. Fifteen animals were incubated under controlled maintenance conditions for 14 days. Five animals were then assayed for CA activity, as described below, which provided values for control symbiotic animal tissue.

Ten anemones were subjected to a low temperature shock, a treatment that rendered them aposymbiotic (Steen and Muscatine, 1987). The anemones were placed in the dark at 4°C, in pre-cooled MFSW, for 4 h and subsequently incubated at 25°C in the dark. As a result of this treatment, *A. pulchella* expelled 99% of its algae within a week. To insure that virtually all of the algae were expelled, these ten anemones were then maintained in the dark at 25°C for ten weeks.

After ten weeks in the dark, five aposymbiotic anemones were placed in the light (12 h light/dark at 40 $\mu E \cdot m^{-2} \cdot s^{-1}$) for repopulation by zooxanthellae. For the first two weeks of the repopulation period, one symbiotic anemone was placed in the bowl with the aposymbiotic anemones as a potential algae donor. After seven weeks, the repopulated anemones had regained their former brown color, indicating the presence of algae and were subsequently assayed for CA activity.

### *Change in CA activity with a change in numbers of algae*

The kinetics of loss of algae and concomitant change in CA activity in the anemone fraction of the association was also quantified. Forty-two anemones were placed, for two weeks, under the control conditions described above. Three anemones were assayed for CA activity and sampled for algal numbers on day one, and another three were kept in control conditions for the duration of the experiment (32 days) and then sampled at the end. These sets were the controls. The remaining anemones were divided into two groups. Half of the anemones were subjected to a cold shock in the same fashion as described above and subsequently maintained in the dark at 25°C. The other half was simply placed in the dark at 25°C (dark treated). Three anemones in each group, cold shock and dark treated, were sampled for algal number and assayed for CA activity after 3, 6, 10, 17, 24, and 32 days in the dark.

### *Separation of algae and anemone tissue for the CA assay*

Anemones were homogenized in a hand-held Teflon-glass tissue homogenizer in 3.5 ml of MFSW chilled to 2°C. The homogenate was transferred to a 10 ml conical centrifuge tube and centrifuged at 900 × *g* for 1 min to separate animal tissue (supernatant) from algae (pellet). There was no evidence that the supernatant was contaminated with algae. The animal tissue supernatant was decanted and diluted 1:1 (v/v) with cold 25 m*M* veronal buffer (2°C), containing 5 m*M* EDTA, 5 m*M* dithiothreitol (DTT) and 10 m*M* $MgSO_4$, adjusted to pH 8.2 (modified from Graham and Smillie, 1976). At this point, the animal tissue supernatant was ready for the CA assay.

Algal pellets were resuspended in MFSW and centrifuged several times, which removed most of the residual anemone debris. The algae were then resuspended in 1 ml of 10% formalin in MFSW, refrigerated and saved. Cell numbers were determined with a haemacytometer and indexed to the weight of soluble anemone protein (determined as described below).

### In vitro *assay for CA activity*

The *in vitro* CA assay is described in detail by Weis *et al.* (1989). The CA activity in animal homogenates was measured by the decrease in pH, resulting from the hydration of $CO_2$ to $HCO_3^-$ and $H^+$, after the addition of substrate. $CO_2$-saturated distilled $H_2O$ served as substrate and was prepared prior to an experiment by passing gaseous $CO_2$ through an air-stone in 200 ml of distilled $H_2O$ at 2°C for 10 min. The water was considered to have been saturated when the pH was below 3.5, and it was then stored in a tightly stoppered glass flask at 2°C.

The assay was run as follows. One milliliter of the buffered animal homogenate was further diluted with 1 ml of 50 m$M$ veronal buffer, (adjusted to pH 8.2 with 1 $N$ NaOH) and transferred to a small glass test tube. The mixture was stirred with a magnetically driven stir bar. One ml of substrate was then added rapidly, and the decrease in pH of the constantly stirred mixture was recorded with a Beckman combination Ag/AgCl pH probe immersed in the mixture and connected to a Beckman Model 45 pH meter. The meter was fitted to an Acorn BBC computer with an analog to digital (A/D) converter that converted the meter output to a digital record. The data were collected and analyzed by a customized software program (John Lighton, copyright 1985).

As a control for non-specific change in pH, the same procedure was carried out with animal homogenate which had been heated to boiling for 5 min, and then cooled to 2°C. This treatment eliminated most or all CA activity. There was no evidence of renaturation upon cooling. CA activity of native animal homogenate and heat-denatured control was measured in triplicate. Units of enzyme activity were normalized to the weight of soluble protein (Hartree, 1972) with bovine serum albumin (Sigma) as a standard. CA activity was expressed as $\Delta$pH units $\cdot$ min$^{-1}$ $\cdot$ mg soluble protein$^{-1}$ as determined from:

$$\frac{(\Delta\text{pH of native animal homogenate} - \Delta\text{pH of denatured control}) \cdot \text{min}^{-1}}{\text{mg soluble animal protein}}$$

*Sample preparation for electrophoresis*

Symbiotic and aposymbiotic anemones were homogenized in a 2.5 ml Teflon-glass tissue homogenizer, in an extraction buffer consisting of 10 m$M$ phosphate buffer at pH 6.8 with 1 m$M$ ethylenediaminetetraacetate (EDTA), 5 m$M$ $MgSO_4$, 5 m$M$ dithiothreitol (DTT), and 2 m$M$ phenylmethyl-sulfonyl fluoride (PMSF), a protease inhibitor. The homogenate was centrifuged at 12,000 rpm in an Eppendorf microfuge for 7 min to pellet the zooxanthellae and animal debris. No evidence was found of contamination of the supernatant by algae. The algal pellet was cleaned three times; in each instance, the cells were suspended and centrifuged in MFSW. The pellet was then stored at −70°C until needed. The slightly milky supernatant, containing the animal tissue, was decanted and stored in a test tube on ice. Usually 8 animals, each with an oral disc diameter of 0.6–0.9 mm, were homogenized in 0.75 ml of buffer to yield a concentration of approximately 4000 $\mu$g protein/ml. Soluble protein was quantified using the method of Hartree (1972).

At least 0.2 ml of packed algae, cultured or freshly isolated, were required to yield enough protein for gel electrophoresis and immunoblotting. For the freshly isolated algae, many frozen pellets from different isolations had to be combined to yield 0.2 ml. The 0.2 ml of thawed algae were suspended in 5 ml of 2% Triton X-100 in MFSW for 10 min to permeabilize and weaken the cell wall and cell membrane. The algae were alternately centrifuged at 2000 rpm in a table top centrifuge, and washed with MFSW, until foam from the Triton was gone from the supernatant. The cells were then resuspended in 0.5 ml of the extraction buffer with approximately 0.3 ml of 425–600 $\mu$m diameter glass beads (Sigma). The mixture was "vortexed" vigorously in a test tube for 1 min and centrifuged, first at 2000 rpm for 1 min in a table top centrifuge, and then at 12,000 rpm for 7 min in a microfuge, to remove the beads, unbroken cells, and cell wall debris. The resulting clear, very deep orange supernatant was decanted and stored in a test tube on ice. This technique disrupted approximately 70% of the cells, as measured by haemacytometer cell counts of samples before and after the treatment, and produced 3500–4000 $\mu$g protein/ml.

Mammalian CA (Worthington Biochemical), used as a control, was dissolved in extraction buffer to a concentration of 500 $\mu$g/ml. Prestained rainbow molecular weight markers (Amersham) were used as standards.

*Electrophoresis and immunoblotting*

Immunoblots, with anti-CA as a probe, were performed on animal tissue and zooxanthellae to determine the nature of CA in the different fractions. SDS-polyacrylamide gel electrophoresis (PAGE) was carried out using techniques modified from Laemmli (1970). A 12.5% resolving gel and a 4.5% stacking gel were most commonly used. Gels, 6.5 cm long and 0.75 mm thick, were run on a Hoefer SE 250 slab gel apparatus with continuous cooling. Before being loaded, the samples were diluted 1:1 with a treatment buffer (Laemmli, 1970) and boiled for 90 s. Twenty $\mu$l of sample were loaded, equalling approximately 40–50 $\mu$g of protein/sample. The gels were run at a constant voltage (200 V) and were stained with either Coomassie blue (Hames and Rickwood, 1987) or silver nitrate (Johnstone and Thorpe, 1987).

Electrophoretic transfer of proteins from unstained gels onto nitrocellulose paper was carried out in a Hoefer TE 22 transfer apparatus for 2 h at 4°C at a constant current (200 mA) in a 25 m$M$ Tris, 192 m$M$ glycine, and 20% methanol buffer, pH 8.3. (Towbin *et al.,* 1979). Subsequently, the nitrocellulose was incubated for 1–2 h in a blocking buffer of 3% Carnation instant dry milk in Tris buffered saline (50 m$M$ Tris, 150 m$M$ NaCl) pH 7.4, and then, overnight, in the appropriate primary antiserum in blocking buffer at room temperature. For each blot, one of two polyclonal antisera was used: a sheep anti-human CA [from Bioproducts for Science (BPS)] at a dilution of 1:200, or a sheep anti-human CA (from ICN) at 1:1000.

The blots were washed in blocking buffer and incubated for 2 h in a 1:1000 dilution of the secondary antibody, an alkaline phosphatase-conjugated, donkey anti-sheep IgG (Sigma). In the development, nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) were used as the substrates (Engvall, 1980; Johnstone and Thorpe, 1987).

## Results

### *CA activity in symbiotic, aposymbiotic, and repopulated anemones*

To determine whether CA activity in animal homogenate is correlated with the presence of algae in animal tissue, CA activity was measured in animal tissue from (1) control symbiotic anemones, (2) aposymbiotic anemones, and (3) repopulated anemones. Both control symbiotic and repopulated symbiotic anemones were light brown and had similar average CA activities of 1.82 ± 0.27 and 1.83 ± 0.40 ΔpH units · $min^{-1}$ · mg $protein^{-1}$, respectively. In contrast, the aposymbiotic animals were white, almost transparent, and had a significantly lower average value of 0.75 ± 0.12 ΔpH units · $min^{-1}$ · mg $protein^{-1}$ (Fig. 1).

### *Change in CA activity with a change in numbers of algae*

To determine whether CA activity would change with a change in numbers of algae, anemones were sampled kinetically, as described above. The number of algae lost with increasing time in the dark was quantified in both cold shock and dark treated anemones (Fig. 2). After just

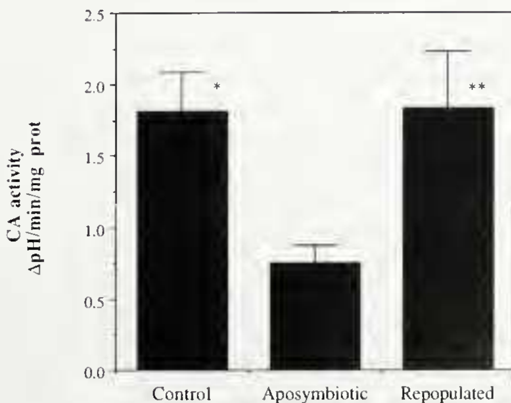


**Figure 1.** CA activity in control anemones, anemones rendered aposymbiotic by cold shock treatment and kept in the dark for 10 weeks, and anemones rendered aposymbiotic, kept in the dark, and subsequently reinfected with zooxanthellae. Each value is a mean ± SD of the mean (n = 5). * = different from aposymbiotic by $P \leq .0001$. ** = different from aposymbiotic by $.0001 < P \leq .005$ as calculated from a one way ANOVA.

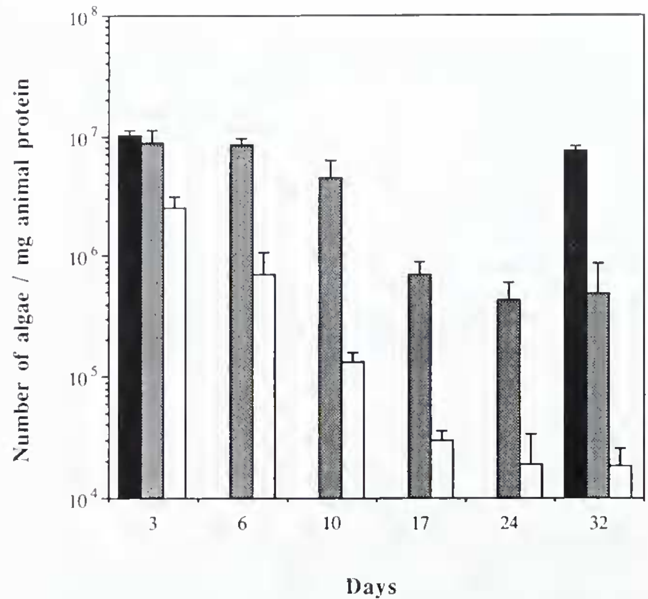


**Figure 2.** Number of zooxanthellae * mg animal $protein^{-1}$ *versus* days in the dark for control ■, dark-treated ▨ and cold shocked □ *Aiptasia pulchella* Each value is a mean ± SD (n = 3).

3 days of darkness, the cold shocked anemones contained less than one third as many algae as the dark treated anemones (Table I). From days 3 to 10, cold shock treated anemones lost over 90% of their algae, compared to only 50% in dark treated anemones. By the end of the 32 day experiment, cold shocked animals had only about 4% of the number of cells/mg animal protein contained in dark treated animals (Table I). The numbers of algae in control anemones, at the beginning and at the end of the experiment, remained high (Table I). Initially, only cold shocked anemones had significantly fewer algae than the controls, but by the end of the experiment both dark treated and cold shocked anemones had lost significant numbers of cells compared to the light controls (Table I).

CA activity in the animal fraction of both dark and cold shock treated anemones was measured with increasing time in the dark (Fig. 3). CA activity in control animals at the beginning and end of the experiment were similar. CA activity in dark-treated anemones decreased modestly, while CA activity in cold shocked anemones decreased more dramatically (Table II). From days 3 to 10, CA activity decreased by 46% in cold shocked anemones, but by only 28% in dark-treated anemones. CA activity in cold shocked *versus* dark-treated animals was significantly different only at day 10 (ANOVA: $.005 < P \leq .01$). Yet at day 32, only CA activity in cold shocked anemones was significantly different (ANOVA) from the control (Table II).

The CA activity in the animal tissue was directly correlated with the number of algae present for both dark

Table I

*Tests for differences in algae numbers between control and treated animals*

| Treatment | # of algae (×$10^6$) animal protein | Control, 3 days 10.20 ± 1.16 | Control, 32 days 7.50 ± 0.72 |
|---|---|---|---|
| Dark, 3 days | 8.72 ± 2.40 | $P > .25$ | — |
| Cold shocked, dark, 3 days | 2.53 ± 0.60 | $.0001 < P \leq .005$ | — |
| Dark, 32 days | .50 ± 0.36 | — | $.0001 < P \leq .005$ |
| Cold shocked, dark, 32 days | .02 ± 0.01 | — | $.0001 < P \leq .005$ |

Significance values from one way ANOVA tests between the listed groups, each with n = 3, are given below along with a mean ± standard deviation for each treatment. The treatment type is listed with the number of days in the dark after the beginning of the experiment.

and cold shock treated anemones (Fig. 4). Most of the lower values were from the cold shock treated anemones.

*Electrophoresis and immunoblotting*

To determine the nature of CA in the association, symbiotic and aposymbiotic anemone tissue, as well as freshly isolated and cultured zooxanthellae, were probed for the presence of CA with polyclonal antisera against human CA. In the immunoblots, both the mammalian CA and symbiotic animal tissue lanes contained one band with an apparent molecular weight of 30 kiloDaltons (kDa) (Fig. 5). One band with an apparent molecular weight of 22.5 kDa appeared in the cultured zooxanthellae lane, and no reaction occurred in the aposymbiotic animal tissue lane (Fig. 5). Freshly isolated algae lanes also contained a single band at 22.5 kDa (data not shown), suggesting that their CA was similar to that in the cultured algae.

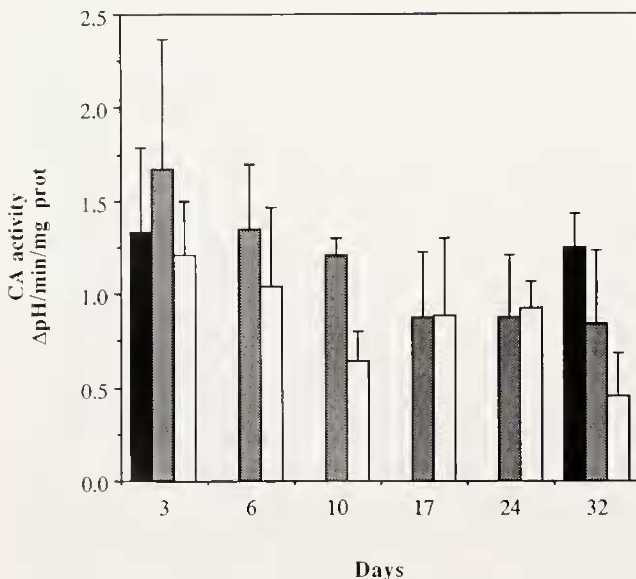


**Figure 3.** CA activity in animal tissue *versus* number of days in the dark for control ■, dark-treated ▨ and cold shocked □ *Aiptasia pulchella*. Each value is a mean ± SD of the mean (n = 3).

The symbiotic animal and cultured zooxanthellae had different relative signal strengths with the two antibodies used (Table III). The symbiotic animal lane gave roughly equal signals at 30 kDa with both the BPS anti-CA and the ICN anti-CA, whereas the algae at 22.5 kDa reacted only with the ICN anti-CA. Both anti-CA probes labeled mammalian CA well.

## Discussion

*Evidence for the correlation of CA activity with the presence of zooxanthellae*

The significant decrease in CA activity in aposymbiotic *versus* control anemones and the subsequent increase in repopulated anemones to control levels (Fig. 1) show that CA activity in anemone tissue is correlated with the presence of algae. These findings are consistent with discovery of a spatial relationship between zooxanthellae and CA activity in the anemone *Condylactis gigantea* (Weis *et al.*, 1989). Additionally, the hypothesis that CA is functioning in the delivery of carbon to the zooxanthellae (Weis *et al.*, 1989) is further supported by these data. Thus, if algae are not present, the supply of $CO_2$ to the anemones requires no augmentation. Although CA activity is low in the aposymbiotic animals, it is not absent. CA is present in virtually all organisms and functions in intracellular pH maintenance (Wyeth and Prince, 1977).

The study of kinetics also reveals a correlation between CA activity and algal numbers. CA activity starts to decrease almost as soon as the cold shocked anemones begin to expel their algae, and it stops decreasing when algal numbers begin to stabilize. The similarity of the CA activity in cold shocked anemones after 32 days (Fig. 3) and ten weeks (Fig. 1) suggests that the decrease in CA activity is discontinued after 32 days. The relatively modest decrease in CA activity over time in dark treated anemones is consistent with the relative paucity of algae expelled from these anemones compared with the cold shocked animal (Fig. 2).

Table II

*Tests for differences in CA activity between control and treated animals*

| Treatment | $\Delta$pH · min$^{-1}$ · mg protein$^{-1}$ | Control, 3 days 1.332 ± 0.457 | Control, 32 days 1.248 ± 0.186 |
|---|---|---|---|
| Dark, 3 days | 1.672 ± 0.694 | $P > .25$ | — |
| Cold shock, dark, 3 days | 1.208 ± 0.288 | $P > .25$ | — |
| Dark, 32 days | .842 ± 0.394 | — | $.10 < P \leq .25$ |
| Cold shock, dark, 32 days | .452 ± 0.237 | — | $.01 < P \leq .025$ |

Significance values from one way ANOVA tests between the listed groups, each with n = 3, are given below along with a mean ± standard deviation for each treatment. The treatment type is listed with the number of days in the dark after the beginning of the experiment.

### *The nature of CA in* A. pulchella

Animal CA, a zinc metalloenzyme, has a molecular weight of approximately 30 kDa, and has as many as six isozymes (Coleman, 1980; Lindskog *et al.*, 1971; Tashian, 1989). Plant CA has been less extensively studied, but occurs in a wide variety of terrestrial and aquatic plants and algae (Lamb, 1977; Poincelot, 1979; Reed and Graham, 1981; Graham *et al.*, 1984). Plant CA varies in molecular weight from about 40 to 250 kDa; it consists of up to 6 subunits ranging in size from approximately 25–34 kDa. Different numbers of subunits and molecular weights have been reported even for a single species (Graham *et al.*, 1984). This study indicates that anemone CA is a 30 kDa protein, whereas CA from freshly isolated or cultured algae is either a 22.5 kDa protein or a protein with several 22.5 kDa subunits (Fig. 5), a weight slightly below the 25–34 kDa range reported for other algae and higher plants (Graham *et al.*, 1984). The successful labeling of both cnidarian and zooxanthellae CA with anti-human CA indicates that at least some portions of the enzyme are highly conserved.

Because protein from freshly isolated algae was difficult to obtain (large quantities of anemones and extensive cleaning were needed to yield enough uncontaminated algal protein), most experiments were performed on cultured algae. The similar labeling of cultured and freshly isolated algae at 22.5 kDa suggests that they have CAs of identical molecular weight.

### *Induction of animal CA by the presence of algae*

Induction or deinduction of an enzyme occurs when the factors controlling its synthetic pathway are removed or changed. Additionally, changes in rates of enzyme degradation can affect the relative activity of an enzyme. These processes can take from minutes to days to be manifested as a change in enzyme activity. Induction of CA activity in the animal tissue in the presence of zooxanthellae could account for the vastly different rates of CA activity in different regions of an individual of *Condylactis gigantea* or in symbiotic *A. pulchella* relative to aposymbiotic ones. In this study, CA activity decreased at a faster rate in the first ten days than in the last 22 in both cold shock and dark treated anemones (Fig. 3). This stabilization of CA activity by the end of the experiment suggests that the putative deinduction or increased degradation of the enzyme took place in the first ten days. A similar plateau in algal population size, although not as well pronounced (Fig. 2), remains consistent with the correlation of CA activity and the presence of zooxanthellae.

The immunoblots of the symbiotic animal tissue, aposymbiotic animal tissue and cultured algae (Fig. 5) suggest that high CA activity in symbiotic animal tissue exhibited in Figure 1 is due to induction of CA in the animal by the presence of the algae, rather than to the presence of algal CA. In Figure 5, anti-CA labeled a single band at 30 kDa in symbiotic animal tissue, whereas no such band appeared in aposymbiotic animals. This result is consistent

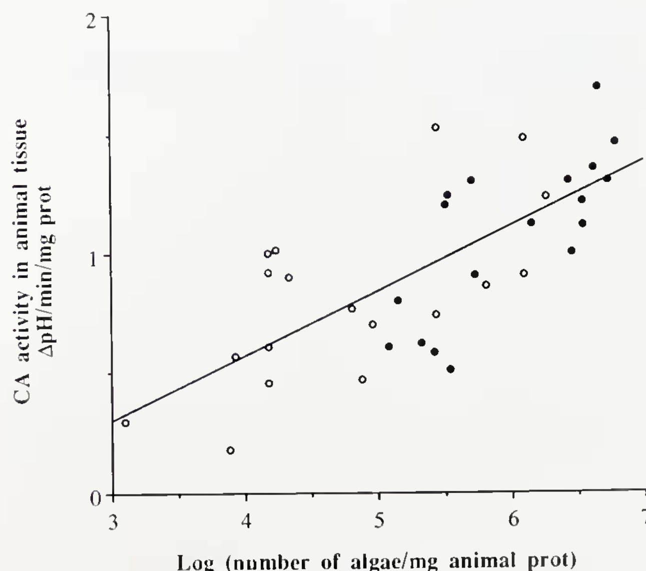


**Figure 4.** CA activity in animal tissue of dark-treated ● and cold shocked ○ anemones *versus* log (number of algae * mg animal protein$^{-1}$). Each point is a datum from a single animal. The $r^2 = 0.518$.

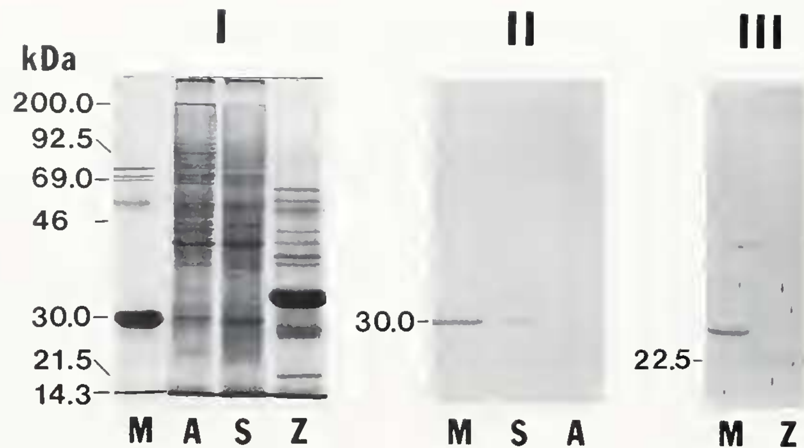


**Figure 5.** An SDS-polyacrylamide gel stained with Coomassie blue (I) and corresponding immunoblots (II and III). Blot II was probed with BPS anti-human CA and blot III with ICN anti-human CA. M = control purified mammalian CA, S = symbiotic animal extract, A = aposymbiotic animal extract, and Z = cultured zooxanthellae extract.

with the loss of enzyme activity in aposymbiotic anemones as compared to symbiotic and repopulated *A. pulchella* (Fig. 1; although there is low CA activity in aposymbiotic anemones, there is not enough protein in the gel to react with the anti-CA probe). The molecular weight of CA from freshly isolated and cultured algae was 22.5 kDa. No band at 22.5 kDa was detected in any symbiotic animal lanes. If algal CA were responsible for CA activity in symbiotic animal tissue, either by export of its CA to the perialgal space or further into the animal tissue, then it should appear on the gel in the symbiotic animal tissue lane.

Induction of CA activity has been studied in detail in mammalian tissues (see Deutsch, 1987, for review), but much less in invertebrates, plants, and algae. Such studies include the induction of CA activity during osmotic stress, to aid in osmoregulation, in various crustaceans (Henry and Cameron, 1983; Wheatly and Henry, 1987; Henry, 1988). CA activity can be induced in some microalgae. The chlorophytes *Chlamydomonas reinhardtii* (*e.g.*, Badger *et al.*, 1978; Coleman *et al.*, 1984, 1985) and *Chlorella vulgaris* (Hogetsu and Miyachi, 1979; Tsuzuki *et al.*, 1980), and the rhodophyte *Porphyridium* spp. (Dixon *et al.*, 1987; Yagawa *et al.*, 1987) show an increase in CA activity when switched from a high to a low $CO_2$ environment.

The mechanism of induction of CA activity is largely undescribed. In humans, the mechanism varies greatly depending on both the function of CA and the tissue or organ type (see Deutsch, 1987, for some examples). As mentioned above, CA is induced in *C. reinhardtii* by low $[CO_2]$ but also by light (Dionisio *et al.*, 1989a, b). In algal/cnidarian symbioses, any number of factors related to presence of algae might induce CA activity in animal tissue, such as increased $[O_2]$ or decreased $[CO_2]$ due to photosynthesis, changes in intracellular pH resulting from different $[CO_2]$, or products, such as glycerol or amino acids, translocated from alga to host (see Cook, 1983, for review).

The kinetics of CA induction or deinduction in animal systems has been studied infrequently. In microalgae, however, the kinetics of CA induction are well described and, in all cases, are shorter in duration than deinduction in *A. pulchella*. Within 24 h after placing *C. reinhardtii* in a low $CO_2$ environment, CA activity increased up to 2000% (Coleman *et al.*, 1984; Badour and Tan, 1987). CA has even been reported to be induced and deinduced at the transcriptional level on a diel cycle in *C. reinhardtii* when the chlorophyte is grown in 12 h light/dark cycle (Toguri *et al.*, 1989).

### *Other examples of induction in symbioses*

There are several algal/cnidarian symbioses in which algae apparently induce enzyme activity or developmental phenomena in the animal. For example, the animal tissue

**Table III**

*Relative signal strengths of the two polyclonal antisera used to probe the experimental samples*

| | ICN anti-human CA 1:1000 dilution | BPS anti-human CA 1:200 dilution |
|---|---|---|
| Purified mammalian CA | +++ | +++ |
| Symbiotic animal tissue | + | + |
| Cultured zooxanthellae | ++ | − |

ICN sheep antiserum was purchased from ICN and BPS sheep antiserum from Bioproducts for Science.

of the symbiotic anemone *Anthopleura elegantissima* contains high levels of superoxide dismutase (SOD) activity compared with SOD in nonsymbiotic anemones. High SOD activity is interpreted as a mechanism for removal of damaging superoxide radicals produced during photosynthesis by the symbiotic algae (Dykens and Shick, 1982, 1984). Also, low molecular weight fractions from homogenates of symbiotic cnidarians suppress uptake of exogenous alanine by isolated zooxanthellae. Similar fractions from aposymbiotic animals fail to suppress uptake (Blanquet *et al.*, 1988). Metamorphosis (which involves complex changes in enzyme expression and activity) of scyphistomae of *Cassiopeia xamachana* and *Mastigias papua*, is induced by the presence of zooxanthellae (Sugiura, 1964; Trench, 1979; Hofmann and Kremer, 1981).

Enzyme induction has also been demonstrated in other symbioses. In the *Rhizobium*/legume symbiosis, the bacteroid nitrogenase activity and host glutamate synthetase activity are positively correlated. Further, the presence of the bacterial symbionts induces the synthesis of the leghemoglobin apoprotein (Smith and Douglas, 1987). In the bacteria/*Amoeba proteus* symbiosis, peribacterial vacuolar membranes contain a protein not found in food vacuolar membranes (Jeon, 1983). Jeon suggests that the synthesis of this protein is induced by the presence of the bacteria, and that the protein somehow prevents lysosomal fusion with the peribacterial vacuolar membrane and subsequent digestion of the bacteria.

This study describes another example of genome interaction between two partners in a symbiosis. Future studies on the molecular mechanisms of induction and regulation of CA should prove fruitful.

## Acknowledgments

I thank S. Anandan and M. Harmon for valuable technical assistance, R. Gates, M. Harmon, G. Somero, and L. Muscatine for comments of the manuscript, and L. Muscatine for valuable suggestions and input. This study was supported by a research grant from the National Science Foundation (OCE-8510518 to L. Muscatine).

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